IN THE CLAIMS:

Please amend the claims as follows:

Cancel claims 10, 63-66 and 103 without prejudice.

REMARKS

1. The Revised Form PTO-1449

On the revised form PTO-1449 returned by the Examiner to Applicants, the Examiner has stricken certain references, stating that no copy was provided and the references were not of record in the parent applications. In addition, certain references on the form PTO-1449 were neither acknowledged as considered nor crossed out by the Examiner. Copies of all of these references are thus submitted herewith, accompanied by a revised form PTO-1449 again listing the references so that the Examiner can indicate his consideration of these references.[1]

2. The Restriction Requirement

Applicant's election with traverse of Group II, claims 60-62, 67-102, and 104-111 is acknowledged by the Examiner. The Examiner has made the restriction requirement final and withdrawn claims 10, 63-66, and 103 from further consideration as drawn to nonelected inventions. Accordingly, Applicants have cancelled claims 10, 63-66 and 103, and reserve the right to prosecute the subject matters of the cancelled claims in one or more related applications.

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[1] Old reference CE now appears as reference HM. Old reference CB is the same as reference AU.

3. <u>The Examiner's Rejection Under 35 U.S.C. § 112, First Paragraph</u>

The specification is objected to, and claims 82-102 are rejected, under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure. The Examiner contends that:

> The invention as defined by pending claims 82-102, is drawn to treatment methods wherein hematopoietic stem cells are administered to a host. However, the specification fails to provide an enabling disclosure for such methods because it fails to provide teachings regarding what such a stem cell would comprise and how one would have obtained and utilized such cells. The term "stem cell" is a generic designation used in the differentiation art to indicate that a given cell is capable of giving rise to a divergent group of progeny cells with variant phenotypic properties. A continuing research effort is aimed at identifying and isolating an "ultimate stem cell" that is capable of giving rise to all of the divergent cell types of the hematopoietic system. The instant specification details the analysis of hematopoietic precursor cells in Section 6.6 and its associated subsections. Said analyses include methods for the identification of CFU-GM, BFU-E and CFU-GEMM progenitor cells (see section 6.6 beginning on page 83). No guidance is present in the specification for the identification, characterization of isolation of any "ultimate" stem cell. However, guidance is present that indicates which groups of cells that may be isolated from whole blood would be useful in the method of the instant invention (see e.g. section 6.3 and associated subsections beginning on page 75). Since the identification of ultimate stem cells is an ongoing field of research and no established guidelines are present in the art for such and further since in the absence of suitable guidance the practitioner would have been required to have exercised undue experimentation in the practice of the claimed invention as currently claimed. Applicant is advised that as pending, the specification fails to provide an enabling disclosure for the invention as claimed. However, should limitation of the extent of the instant invention to utilization of particular enriched fractions of blood which include identifiable and assayable populations of cells would obviate the instant grounds of objection and corresponding rejection of the claims.

Applicants respectfully disagree with the Examiner's rejection. The rejected claims (directly or indirectly via claim dependency) recite "blood components comprising hematopoietic stem cells." The specification teaches in detail how to obtain such components and how to test such components for the presence of hematopoietic stem cells.

Preliminarily, Applicants submit that, as is clear from the instant specification, the "ultimate" stem cell is the cell which can be identified functionally by its ability to provide hematopoietic reconstitution (*i.e.*, long-term, complete (multilineage) marrow repopulation). Applicants were the first to conceive and teach that human neonatal and fetal blood contains the long-term marrow repopulating cell in sufficient amounts to effect hematopoietic reconstitution. Applicants also were the first to conceive and teach that fractions of human neonatal/fetal blood containing types of stem cells such as were assayable by other methods as of the filing date, *e.g.*, by spleen colony formation (CFU-S) or the ability to generate progenitors for secondary colonies, also generally contained the long-term marrow repopulating type of stem cell, and thus have use for hematopoietic reconstitution and therapy of disorders in which hematopoietic reconstitution is therapeutically effective. Applicants have provided ample guidance in the specification teaching various methods for enrichment and/or purification of blood stem cells (which, based on Applicants' teachings, generally include the long-term marrow repopulating stem cell in the blood fraction) (see Section 5.1.3.1 of the specification, pages 37-43); this, in view of knowledge common in the art, shows that there is no justification for limiting the claims to any particular enriched fraction of blood.

Applicants teach in detail how to obtain and assay "blood components comprising hematopoietic stem cells" as recited by the claims. The Examiner's attention

is invited to the instant specification, Section 5.1.3.1, pages 37-43, entitled "Enrichment for Hematopoietic Stem and Progenitor Cells: Cell Separation Procedures," wherein numerous procedures which can be used to obtain populations of cells enriched in hematopoietic stem cells are described.

Assays to screen purified or enriched cell populations for the presence of stem cells are provided by the specification (see *e.g.*, Section 5.4.2, pages 49-50, regarding CFU-S and S cell assays; see also Section 2.1, pages 6-9). Other assays known in the art may also be used. Thus, one skilled in the art can readily, without undue experimentation, obtain and identify the populations of cells used in the claimed methods.

Moreover, Applicants point out that there are no reasonable grounds of record to doubt that human neonatal or fetal blood components comprising hematopoietic stem cells can be readily isolated as taught by Applicants. A patent applicant's specification disclosure which contains a teaching of how to make and use the invention must be taken as enabling unless the Patent Office provides sufficient reason to doubt the accuracy of the disclosure. In re Marzocchi, 439 F.2d 220, 223-24 (C.C.P.A. 1971). Thus, the specification, combined with knowledge common in the art, provides sufficient guidance to enable the claimed methods of treatment comprising isolation of blood components comprising human hematopoietic stem cells as recited by the claims. The Examiner's rejection is in error and should be withdrawn.

5. The Examiner's Rejection Under 35 U.S.C. § 103

Claims 60-62, 67-102 and 104-111 are rejected under 35 U.S.C. § 103 as being unpatentable over Nakahata et al., 1982, J. Clin. Invest. 70:1324-1328 (reference DX); Saunders, 1965, U.S. Patent No. 3,177,117 (reference AN); and either of Ende, 1966, Pac. Med. & Surg. 74:80-82 (reference BV) or Ende et al., 1972, Va. Med.

Monthly 99:276-280 (reference BU); in view of Applicants' alleged admissions on pages 10, 11, 27 and 28; Herzig et al., 1983, *in* Bone Marrow Transplantation, Weiner et al., eds., The Committee on Technical Workshops, American Association of Blood Banks, Arlington, Virginia (reference CQ); McGlave et al., 1985, *in* Recent Advances in Hematology, Hoffbrand, A.V., ed., Churchill Livingstone, London, pp. 171-197 (reference DT); and Fabian et al., 1982, Exp. Hematol. 10:119-122 (reference BW). According to the Examiner:

> The instant invention is directed towards methods of treating diseases involving depletion of hematopoietic cells by administration of cryopreserved neonatal or fetal blood cells to a patient.
>
> Each of Ende (BV) and Ende (BU) teaches the treatment of patients with human fetal (cord) blood. In the case of Ende (BV), the patient suffered from leukopenia and general anemia and was treated with human umbilical cord blood (see e.g. Table I, page 81). In the case of Ende (BU) the patient suffered anemia due to conventional antileukemic therapy (see e.g. introduction on page 276). Neither of Ende (BV) or Ende (BU) teaches the cryopreservation of fetal cord blood or the treatment of the variety of anemic disorders as instantly claimed.
>
> Nakahata teaches that human fetal cord blood is comprised of a variety of hematopoietic progenitor cells that are similar in composition to those present in adult bone marrow (see e.g. Table I, page 1326).
>
> Each of Herzig and McGlave teach the utilization of bone marrow transplantation (BMT) for the treatment of disorders associated with anemia. Herzig specifically discloses the use of BMT and that bone marrow contains a variety of hematopoietic precursor cells (see e.g. section entitled Viability beginning on page 125). Herzig also teaches the use of cryopreserved bone marrow in therapeutic applications (see e.g. section entitled Cryopreservation beginning on page 126). McGlave discloses both allogeneic and autologous BMT for treatment of a variety of disorders (see e.g. section entitled Clinical Applications of Bone Marrow Transplantation, beginning on page 171).

Fabian and Saunders disclose cryopreservation of multipotential hematopoietic cells (see e.g. Fabian; Abstract and Table I on page 121 and Saunders; column 2, lines 46-62). Saunders additionally discloses the use of mannitol as a cryoprotectant that may be administered concomitantly with cryopreserved blood.

Applicants admit within the body of the paragraph bridging pages 10 and 11 that bone marrow has been used with increasing success for treatment of a variety of disorders and further admit on page 27 that it was known that human cord blood contains a high proportion of hematopoietic precursor cells.

As indicated above, human cord blood and bone marrow cells have similar properties in that they both comprise significant populations of hematopoietic progenitor cells. Therefore, since the cellular components of these two compositions were known to be similar, one would have had a reasonable expectation of success in utilizing cord blood and its components in situations where bone marrow cells were employed. It was recognized that cryopreservation was a useful method for preserving cells prior to administration and additionally Saunders explicitly noted that certain cryoprotectants could be co-administered with blood without inducing adverse effects. Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have utilized cryopreserved cord blood cells in methods of treating any disorder in which the use of bone marrow cells would have been appropriate.

One would have been motivated to use fetal blood cells in a treatment method because it was recognized that such cells contained high proportions of hematopoietic progenitor cells which the artisan would have recognized as useful in the treatment of a variety of disorders characterized by anemia. One would have used cryopreserved blood cells because one would have expected that by preserving blood, one would have increased the storage of life of blood to be used in future applications.

In regard to claims 82-102, it is noted that the instant grounds of rejections is applicable in so far as said claims could be considered to comprise the use of hematopoietic precursor cells rather than the use of the ultimate hematopoietic stem cell.

> Therefore, for the preceding reasons, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Applicants respectfully disagree. Applicants submit that the instant claims are not rendered obvious by the combination of references as set forth above, for reasons discussed in detail below.

A. The Legal Standard for Obviousness

The claimed invention is not rendered obvious by the cited art using the objective standard for obviousness under 35 U.S.C. § 103 set forth clearly by the Supreme Court of the United States in Graham v. John Deere, Inc., 383 U.S. 1 (1966). According to this Supreme Court decision, the Examiner is required to ascertain: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; and (3) the differences between the claimed subject matter and the prior art. *See* 383 U.S. at 17. The obviousness or non-obviousness of the claimed subject matter must be determined in light of these inquiries. Following Graham, the Court of Customs and Patent Appeals (C.C.P.A.) and its present successor, the Court of Appeals for the Federal Circuit (CAFC), have held the following considerations to be objective evidence of nonobviousness: long felt need, commercial success, failure of others, copying and unexpected results. *See* In re Sernaker, 702 F.2d 989, 217 U.S.P.Q. 1 (Fed. Cir. 1983); In re Imperato, 486 F.2d 585, 179 U.S.P.Q. 730 (C.C.P.A. 1973).

A rejection for obviousness is improper when there is nothing in the cited prior art references, either singly or in combination, to suggest the desirability of the claimed subject matter. For a rejection of claimed subject matter as obvious in view of a combination of prior art references to be upheld, (1) the prior art must have suggested to those of ordinary skill in the art that they should make the claimed composition or device

or use the claimed method, as the case may be; and (2) the prior art must have revealed that in so doing, those of ordinary skill would have had a reasonable expectation of success. In re Vaeck, 947 F.2d. 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991); In re Fine, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988); In re Dow Chemical Co., 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Furthermore, Applicants submit that the rejection of the instant claims under § 103 indicates the improper use of hindsight gained from Applicants' own specification. Hindsight should be avoided in applying the nonobviousness requirement. Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1 U.S.P.Q.2d 1593 (Fed. Cir. 1987), cert. denied, 481 U.S. 1052 (1987). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." In re Fine, 837 F.2d 1071, 1075, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988).

Without the benefit of hindsight, the teachings of the references cited by the Examiner, alone or in combination, could not possibly render obvious the claimed invention. The claimed methods could not have been foreseen by a person of ordinary skill in the art, since there was no suggestion of them in the art and their utility could not have been predicted (see discussion below). A finding of obviousness could only be arrived through a prohibited procedure in which "the claims were used as a frame, and individual naked parts of separate prior art references were employed as a mosaic to recreate a facsimile of the claimed invention." W.L. Gore & Assocs. Inc. v. Garlock, Inc., 721 F.2d 1540, 1552, 220 U.S.P.Q. 303, 312 (Fed. Cir. 1983). cert. denied, 469 U.S. 851 (1984).

B. A Brief Summary of Applicants' Position

Applicants point out that, as described in the instant application, Section 2.1, hematopoietic stem and progenitor cells are the cells from which the mature functional cells circulating in the blood derive. Stem cells are the most primitive cells in the hematopoietic lineage; they have extensive proliferative capacity and the ability to generate other stem cells as well as to differentiate into the progenitor cells, which in turn can differentiate into the mature cells. The mature cells include erythrocytes (red blood cells), granulocytes, monocytes/macrophages, megakaryocytes, T cells, B cells, and non-T, non-B cells. The term "hematopoietic reconstitution" as used herein and in the instant application, refers to long-term, complete reconstitution (repopulation) *in vivo* of the multiple cell lineages which make up the blood (myeloid (including erythroid components)) (see instant specification at page 9, lines 17-18, 28-34; page 24, lines 17-24). It is the stem cell with long-term marrow repopulating ability, that in sufficient amounts has utility for hematopoietic reconstitution. Bone marrow transplants attempt to achieve hematopoietic reconstitution. This is in contrast to blood replacement, which is routinely afforded by blood transfusions using noncryopreserved blood (such as is found in blood banks).

Applicants point out that none of the cited references motivate the therapeutic use of a composition comprising human neonatal or fetal blood stem cells derived from the blood that have been cryopreserved or that are in combination with a cryoprotective agent. For the reasons discussed in detail below, Applicants emphasize that teachings of the prior art regarding bone marrow stem cells cannot be applied to render the instant invention obvious because one of ordinary skill in the art would not reasonably expect hematopoietic stem cells from different sources (*e.g.*, from neonatal/fetal blood as opposed to bone marrow) to have the same properties concerning

therapeutic utility in the absence of Applicants' teachings. As pointed out in the instant specification on page 24, lines 1-2, neonatal blood obtained from the umbilical cord and placenta was deemed in the prior art to be so lacking in utility that it was customarily discarded at birth. It was the inventors of the instant application who discovered the utility of such neonatal/fetal hematopoietic stem cells of the blood. Based on this discovery, applicants invented the methods defined by claims 60-62, 67-102 and 104-111.

The combined teachings of the cited references do not give rise to such a reasonable expectation of therapeutic utility of human neonatal or fetal blood stem or progenitor cells that have been cryopreserved because, in the absence of the teachings of the instant application, one of ordinary skill would not by analogy apply the therapeutic utility for hematopoietic reconstitution of cryopreserved bone marrow cells (*e.g.* as taught by Herzig) to cryopreserved human fetal/neonatal blood stem cells. This is true even though it was known that certain hematopoietic cells existed in cord blood as identified by colony forming assays, without any replating (progenitor cells) or even cells as might be identified by the ability to give rise to colonies that can be replated to form secondary colonies (*e.g.*, as taught by Nakahata et al.). One of ordinary skill would not have a reasonable expectation that a composition comprising human neonatal/fetal blood stem (or progenitor) cells can be cryopreserved and used to treat various diseases and disorders by virtue of its ability to effect hematopoietic reconstitution, for several main reasons: (1) knowledge common in the art regarding prior art sources of stem cells would, if anything, have led one of ordinary skill in the art to doubt the utility of human neonatal or fetal stem cells of the blood for hematopoietic reconstitution (see discussion *infra* and Second Bernstein Declaration); (2) the hematopoietic repopulating cell (*i.e.*, the "stem cell" with utility for *in vivo* reconstitution) would not be expected to be identical with a cell identified by the ability to generate colonies *in vitro*, before or even after replating (see

also the Second Bernstein Declaration ¶ 21); (3) even assuming *arguendo* that the cell with *in vivo* reconstituting ability was present, there is <u>no</u> teaching or expectation in the art that a collection of human neonatal/fetal blood cells that had been cryopreserved, particularly from a single human neonate or fetus, would have <u>sufficient</u> amounts of stem cells to be therapeutically useful; and (4) concerns of maternal cell contamination and graft-versus-host disease would have prevented an expectation of utility. Further, the claimed invention exhibits secondary indicia of nonobviousness, in particular, fulfillment of a long-felt need in the art, copying by others, and the achievement of surprising results. The above reasons are discussed in detail below. To document their position, Applicants rely on several sources. Specifically, support is found in the references relied upon by the Examiner, other publications in the art, and the Second Declaration of Dr. Irwin D. Bernstein (which was submitted in connection with copending Reexamination No. 90/003182 filed August 30, 1993 of co-owned U.S. Patent No. 5,004,681).[2]

C. <u>The References Relied Upon by the Examiner</u>

Applicants provide the following comments regarding the teachings of the references cited by the Examiner.

Nakahata et al. (reference DX) teaches the presence in human cord blood of cells which form blast cell colonies *in vitro* which can be replated to form secondary colonies, including multipotential secondary colonies. The authors state that they failed to identify blast cell colonies in culture of human adult marrow or peripheral blood cells (p. 1328, col. 1), and that "[f]urther improvement of the replating conditions is necessary

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2 Although the claims at issue in Reexamination No. 90/003182 are directed to compositions comprising human neonatal/fetal stem cells derived from the blood, and cryopreservative, the propositions for which the Second Bernstein Declaration are cited herein are also applicable to the instantly claimed invention.

for confirmation of the self-renewal capacity of human blast cell colonies" (p. 1328, col. 1).

Saunders et al. (reference AN) discloses a process for cryopreservation of blood, the use of mannitol as a cryopreservative, as well as the clinical transfusion of mannitolized blood. There is no disclosure or suggestion of the use of human neonatal or fetal blood.

Ende (reference BV) describes the administration of human umbilical cord blood to a patient with lymphangiosarcoma. The author states that the administration of cord blood was undertaken because such blood may contain factors which inhibit neoplasms. Cord blood from 17 different deliveries was administered over a period of almost one month, from which the patient "seemed to derive some temporary benefit." There was no cryopreservation of cord blood prior to transfusion, or any suggestion thereof. In fact, "[t]he object was to give the blood to the patient as quickly as possible following its collection in the delivery room under sterile conditions" (p. 82). There is no evidence indicating the utility of cord blood for hematopoietic reconstitution.

Ende et al. (reference BU) discloses mere transfusions using human cord blood. Ende et al. (BU) describes an attempted treatment of a patient with acute lymphoblastic leukemia by the transfusion of a total of eight human umbilical cord blood samples from different donors, over a period of 17 days. There was no cryopreservation of cord blood prior to transfusion. Ende et al. does <u>not</u> report a successful hematopoietic reconstitution since only a brief, temporary change in the patient's red blood cell (erythrocyte, a mature cell) phenotype was observed. See Second Bernstein Declaration ¶¶ 31-32. In fact, Ende et al. provides no reasonable basis for believing that any hematopoietic reconstitution was achieved or that any therapeutic effect was achieved which would motivate cryopreservation of a composition comprising cord blood stem cells

or therapeutic use of such a cryopreserved composition. In fact, the transient change in blood antigen which was observed was limited to red blood cell antigens, was for a brief time period,[3] and would be attributed by one of ordinary skill in the art to red blood cells present in or produced from progenitor cells originally present in the transfused blood (see Second Bernstein Declaration, ¶ 31). Ende et al. does not even come close to giving one skilled in the art a reasonable expectation that hematopoietic reconstitution was attained, or even that viable hematopoietic stem cells capable of marrow repopulation were present in the transfused blood, because the recipient in no way exhibited reconstitution of hematopoietic function that is long-term or broad (multilineage, including the lymphopoietic system) and thus indicative of long-term marrow-repopulating stem cell function (Second Bernstein Declaration, ¶ 31). Ende et al. itself states that "in this case it is uncertain as to whether the recipient had a transplant of the lymphoid elements" (page 4, column 2). Furthermore, Ende et al. admit that the remission obtained in the patient was "not necessarily related" to the administration of cord blood (p. 1, col. 2).

Short-term reconstitution of red blood cells such as is disclosed by Ende et al. (reference BU) does not motivate one to cryopreserve cord blood, since routine transfusions of noncryopreserved blood (*e.g.*, from blood banks) routinely fulfill any need for red blood cell replacement (see Second Bernstein Declaration, ¶ 31). Thus, Ende et al. does not provide any motivation to cryopreserve a composition containing cord blood stem cells, much less to use such a cryopreserved composition therapeutically.

As further evidence of the foregoing, the Examiner's attention is directed to an article dated July 6, 1995, "Transplantation of Cord-Blood-Cells," N. Engl. J. Med.

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3 Administration of blood from donor M occurred on March 7, 1970; donor M antigen in the recipient appeared and peaked from March 11, 1970 through April 14, 1970, disappearing by May 13, 1970.

333(1):67-69 (reference GG) ("July Article"). The July Article presents letters to the Editor regarding an editorial on cord blood cell transplantation by Dr. Robert P. Gale, M.D., Ph.D. of Salick Health Care, Inc., Los Angeles, which appeared in the February 9, 1995 issue of the New England Journal of Medicine, Vol. 332, pp. 392-394 (reference GU).[4] In response to Dr. Gale's editorial, Drs. Mark Ende and Frederick Ende wrote a letter to the Editor, bringing Ende et al., 1972, Va. Med. Monthly 99:276-80 (reference BU, cited by the Examiner as a basis for the § 103 rejection of the claims of the instant application) to the attention of the Editor, stating as follows (1995, N. Engl. J. Med. 333:68, col. 1):

> *To the Editor:* Dr. Gale's editorial contains an error. He states, "The only successful cord-blood transplantations have been performed in small children." A report published in 1972 described the use of umbilical-cord blood as a source of hematopoietic stem cells for transplantation in a 16-year-old patient with leukemia.[1]
>
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>
> 1. Ende, M, Ende N. Hematopoietic transplantation by means of fetal (cord) blood: a new method. Va Med Mon 1972;99:276-80.

In response to this letter by Drs. Ende and Ende, and in clear confirmation of the position taken by Applicants and Declarant Dr. Irwin Bernstein, Dr. Gale replies as follows (1995, N. Engl. J. Med. 333:69, col. 1):

> Drs. Ende and Ende report on a young man with leukemia who received infusions of cord-

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4 Gale, February 9, 1995, "Cord-Blood-Cell Transplantation -- A Real *Sleeper*?" N. Engl. J. Med. 332(6):392-394 (reference GU) is the editorial to which the July Article refers, and is submitted with the accompanying Information Disclosure Statement for purposes of completeness of the record.

> blood cells from several donors after chemotherapy. HLA typing was not performed for any of the grafts, and the chemotherapy was mild by transplantation standards. I would not expect engraftment to occur under these conditions and am not convinced by the data reported, which relied on red-cell typing rather than HLA typing or cytogenetic analyses.[4] [footnote 4: Ende and Ende, 1972, Va. Med. Monthly 99:276-80 ("Ende et al.")] (emphasis added).

In confirmation of Applicants' position, the July Article shows that Dr. Gale, like Dr. Bernstein, another disinterested third party, views the provision of blood components by temporary transfusion, as taught by Ende et al. (BU), not as provision of stem cell function, but, rather, as provision of mature cells (red blood cells). Since stem cells are not implicated, there is no motivation to store compositions comprising cryoprotective agent and stem cells derived from cord blood or from other neonatal/fetal blood, or to use such compositions therapeutically. For purposes other than hematopoietic reconstitution, human adult peripheral blood was abundant, available, and replenishable, such that there was no motivation to use the relative unabundant and inaccessible human neonatal or fetal blood.

Herzig (reference CQ) teaches bone marrow transplantation for hematopoietic reconstitution, including the use of cryopreserved bone marrow. Herzig also teaches that there are no suitable assays for the long-term marrow repopulating stem cells that effectuate hematopoietic reconstitution. Herzig discloses that *in vitro* colony assays of committed hematopoietic precursor cells "such as the granulocyte-macrophage colony-forming assay (CFU_c) or erythrocyte assays (. . . BFU_E; . . . CFU_E)" detect committed cells which differ from the pluripotent stem cell (p. 125). Herzig further discloses that CFUs are pluripotent stem cells, detected by the spleen focus forming assay in mice, and then goes on to state:

> While these CFUs are more primitive than committed precursors, it remains uncertain whether all stem cells that form spleen colonies are also capable of restoring bone marrow function after transplantation. The same uncertainty exists for the more recently developed human pluripotent stem cell assay.[21]
>
> The only unequivocal test of stem cell preservation is the demonstration of the ability of transplanted marrow to restore hematopoiesis after a treatment that produces permanent marrow aplasia. (p. 125)

There is no hint or suggestion in Herzig of stem cells from human fetal or neonatal blood.

McGlave (reference DT) discloses allogeneic and autologous bone marrow transplantation for the treatment of a variety of disorders. Graft-versus-host disease is disclosed to be a major concern (pp. 180-189). There is no hint or suggestion of stem cells from human fetal or neonatal blood.

Fabian (reference BW) teaches cryopreservation of multipotential hematopoietic cells from human bone marrow. (The cells are detected by colony-forming assays (in the absence of replating)). There is no hint or suggestion of stem cells from human fetal or neonatal blood.

In view of the above remarks, it is clear that none of the cited references, alone or in combination, suggest with a reasonable expectation of success the therapeutic use of compositions comprising human neonatal/fetal blood-derived stem cells that have been cryopreserved (or that are in combination with a cryoprotective agent). Additional remarks demonstrating the nonobviousness of the claimed invention follow in the subsections below.

D. The Claimed Invention Is Not Obvious

Knowledge common in the prior art regarding human bone marrow, adult peripheral blood, and fetal liver as sources of stem cells for hematopoietic reconstitution would, if anything, have led one of ordinary skill in the art to doubt the utility of human neonatal or fetal stem cells of the blood for hematopoietic reconstitution, and thus provides no motivation to cryopreserve such cells or to treat diseases amenable to therapy by hematopoietic reconstitution by administering cryopreserved human neonatal or fetal stem cells of the blood. As explained by Dr. Bernstein in ¶ 30 of the Second Bernstein Declaration (copy submitted herewith), it was known that in the human adult, sufficient amounts of long-term marrow-repopulating stem cells sufficient to provide utility for hematopoietic reconstitution did not normally circulate in the blood; adult peripheral blood was generally believed useful only autologously when "rebounding after chemotherapy" (*i.e.*, undergoing in cancer patients a chemotherapy-induced overshoot of the normal stem and progenitor cell levels). In contrast, adult bone marrow was viewed as the stem cell-containing "generating center" for the blood in the adult. Similarly, fetal liver was viewed as the stem cell-containing "generating center" for the blood in the fetus. Thus, assuming *arguendo* that the use of neonatal or fetal blood as a source of stem cells for hematopoietic reconstitution had been proposed to one of skill in the art, by analogy to the situation in the adult, it would have been doubted that sufficient amounts of long-term marrow-repopulating stem cells circulated in fetal or neonatal blood so as to provide utility for hematopoietic reconstitution, since the stem cells were expected to stay relatively concentrated in the "generating center."

As Dr. Bernstein states (Second Bernstein Declaration, ¶ 30):

> assuming for the sake of argument, that the use of human neonatal/fetal blood as a source of stem cells for hematopoietic reconstitution had been suggested to me, the fact that normal adult peripheral blood was believed to have such a low content of long-term marrow repopulating stem

cells relative to that present in bone marrow,[5] would if anything, have led me to expect that normal neonatal or fetal blood would have similarly low levels of long-term marrow repopulating stem cells and thus would not be likely to have utility for hematopoietic reconstitution. For the reasons I explained in Paragraphs 9-20 above, knowledge of cord blood cells which exhibit colony-forming abilities *in vitro*, even after replating, does not provide any information regarding the presence or quantity of long-term marrow repopulating stem cells, particularly since the ratio of stem cells (and in particular the long-term marrow repopulating stem cells) to progenitor cells in human neonatal/fetal blood is unknown.[6] At about the time the application leading to the '681 Patent[7] was filed, I viewed fetal liver and adult bone marrow as being similar in that both were known to be "generating centers" for blood components, of the fetus and adult, respectively, and thus it was perhaps not unexpected in hindsight that both contained long-term marrow repopulating stem cells such that they could be used as sources of these cells for hematopoietic reconstitution. Since it was known that normal adult peripheral blood had relatively low levels of progenitor cells, and that success in using adult peripheral blood cells for hematopoietic reconstitution had generally been attained only in situations where a leukemia patient's blood expanding in response to chemotherapy was employed, this indicated to me, and I believe also to others in the art, that adult long-term marrow repopulating stem cells generally did not normally circulate in the blood, but, rather, stayed localized in the generating center, *i.e.*, bone marrow. By similar reasoning, I would not have expected that fetal or neonatal long-term marrow repopulating stem cells circulated in neonatal or fetal blood, much less in amounts sufficient to

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5 Dr. Bernstein refers by footnote here to Paragraph 29 of the Second Bernstein Declaration.

6 Dr. Bernstein includes two footnotes here, respectively stating: "It was clearly unknown at about the time of the filing of the application leading to the '681 Patent. As of 1989, it was still unknown: As Linch and Brent (1989, Nature 340:676) state: 'A 100-ml sample of cord blood can be expected to contain . . . progenitor cells, which should be sufficient for reconstitution after allogeneic transplantation [citation] provided that the stem-cell/progenitor-cell ratio is not appreciably less than in adult bone marrow. Only clinical studies can prove this point.' To my knowledge, even as of the present date, the ratio is still unknown." and "The ratio of long-term marrow repopulating stem cells to other cells designated 'stem cells' (based on their detection by assays other than the ability to effect hematopoietic reconstitution) was and is also unknown."

7 The '681 Patent is U.S. Patent 5,004,681, issuing from application Serial No. 119,746, to which the instant application claims priority.

afford utility for hematopoietic reconstitution;[8] instead, I would have expected that such cells stayed localized in the generating center, *i.e.*, fetal liver. Indeed, had the idea of using human neonatal or fetal blood as a source of cells capable of effecting hematopoietic reconstitution in humans been disclosed to me at about the time the application leading to the '681 Patent was filed, I would have been skeptical that this idea would work. However, now that the utility of human cord blood as a source of stem cells that can be cryopreserved and used to effect hematopoietic reconstitution has been proven by many clinical successes in children having various disorders, I would not hesitate in certain situations to use cord blood stem cells for hematopoietic reconstitution, or to recommend such use of cord blood stem cells by others in the art, for example, in young children suffering from genetic disorders amenable to treatment by hematopoietic reconstitution. I believe that one of ordinary skill in the art at about November 1987 would have reasoning and conclusions substantially the same as mine set forth hereinabove in this Paragraph.

Additionally, numerous publications evidence that different sources of stem cells were not deemed interchangeable by one of ordinary skill in the art at the time the invention was made, with respect to their utility for hematopoietic reconstitution. For example, Herzig (1983, "Autologous bone marrow transplantation," in *Bone Marrow Transplantation*, Ch. 6, Weiner et al. (eds.), The Committee on Technical Workshops, American Association of Blood Banks, Arlington, Virginia, pp. 123-146) (reference CQ) (cited by the Examiner as a basis for the instant § 103 rejection), after discussing the relative advantages and disadvantages of autologous and allogeneic bone marrow

8 Dr. Bernstein includes a footnote here, stating: "It will thus be even more evident that I would not reasonably have expected that amounts of long-term marrow-repopulating stem cells sufficient to confer utility for hematopoietic reconstitution would be present in any single collection of human neonatal/fetal blood (*i.e.*, from a single individual); nor would one of ordinary skill in the art reasonably have expected this. Blood collected from only a single neonate or fetus would have been deemed necessary for use of human neonatal/fetal blood for hematopoietic reconstitution (except in rare circumstances such as the case of collection from identical twin neonates or fetuses), since combinations of collections from different neonates/fetuses would be avoided due to the danger of graft versus host disease and other problems stemming from histocompatibility mismatches, contamination by maternal cells, infectious agents, etc."

transplantation (pp. 123-124), mentions encouraging results with the use of autologous (self) adult peripheral blood only in patients with chronic myelogenous leukemia, (pp. 124-125), "a condition in which the peripheral blood is thought to be greatly expanded" (p. 125), and that this has been followed by subsequent failures, yielding dubiousness and great uncertainty regarding the utility of adult peripheral blood for hematopoietic reconstitution. As yet another example, Raghavachar et al., 1983, J. Cell Biochem. Suppl. 7A:78 (Abstract 0198) (reference EI), explicitly state (in the context of comparing cryopreserved adult peripheral blood mononuclear cells and bone marrow from dogs) that "[o]ur results are in agreement with the hypothesis that there are differences in the relationship between CFU-c [disclosed in this reference to be hemopoietic progenitor cells] content and hemopoietic potential of autografts from different sources." Gorin, 1986, Clin. Haematol. 15:19-48 (reference CL), notes that "[n]umerous experimental studies indicate that in animals peripheral blood stem cells are physiologically different from marrow stem cells and the question remains unsolved in man" (p. 32, fourth paragraph).

Knowledge that bone marrow contains therapeutic amounts of stem cells does not indicate the presence of human neonatal or fetal blood stem cells with therapeutic utility because knowledge of the existence of long-term marrow repopulating stem cells from sources other than human neonatal or fetal blood does not yield any information on whether human neonatal or fetal blood contains such stem cells much less in therapeutic amounts. In support of this, the Examiner's attention is directed to Thompson, 1995, "Umbilical Cords: Turning Garbage Into Clinical Gold," Science 268:805-806 (reference IB) ("Thompson"), which shows that cord blood stem cells were considered garbage prior to the invention of the instant application. Thompson describes how the scientific and medical community formerly viewed cord blood as "garbage" until

the idea of using cord blood stem cells for hematopoietic reconstitution and for gene therapy (provided by the instant inventors) started changing such "garbage" into "clinical gold." As Thompson states, "[w]hat was a discard has become valuable -- indeed priceless to many children with leukemia . . . " This article clearly shows that prior to the disclosure of the invention of the instant application there was no motivation to freeze cord blood stem cells, much less use them therapeutically, and that it was the inventors of the instant application that provided the first motivation to freeze and then administer such cells, "turning garbage into clinical gold." The article demonstrates that there was no reasonable expectation of success of the claimed methods in the prior art.

Indeed, even though it was known that cord blood contained cells which were characterized by the ability to form colonies of different hematopoietic cells *in vitro* upon replating, such cells are not deemed identical to the hematopoietic reconstituting stem cell, and thus one would not reasonably expect that human neonatal/fetal blood containing such cells with colony-forming ability would be able to carry out human hematopoietic reconstitution in the absence of Applicants' teachings or clinical evidence *in vivo* of such ability. See Second Bernstein Declaration ¶ 21. The foregoing statement is also evidenced by publications previously made of record, as well as Herzig. (The Examiner is referred to the discussion of Herzig hereinabove.) Williams et al., 1987, Nature 310:476-480 (reference GB) discuss the "S-cell," which is stated to be the secondary colony-forming cell identified *in vitro* (from cord blood) by Nakahata and Ogawa in, *inter alia*, the Nakahata et al. reference cited by the Examiner as a basis for the instant rejection. Williams et al. disclose that "[i]t is the PHSC [pluripotential hematopoietic stem cell] which provides long-term repopulation of the hematopoietic organs following bone marrow transplantation" (p. 294), and then note that "[n]o evidence is yet available regarding the possible lymphoid differentiation potential of the

S-cell making the relationship between the PHSC and the S-cell unclear" (p. 296). In the publication by Broxmeyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:3828-3832 (reference BD) it is stated that "there is not yet a direct assay for human hematopoietic repopulating cells" (p. 3831, col. 2), and in the publication by Broxmeyer et al., 1990, Int. J. Cell Cloning 8(Suppl. 1):76-91 (reference BA), it is stated that the "major problem with assessing the numbers of hematopoietic repopulating cells that could be isolated from umbilical cord blood was the lack of a quantitative assay for these cells in humans" (p. 78). Similarly, Moritz et al, 1993, J. Exp. Med. 178:529-536 (reference DW) state that "no direct in vitro assay is available to determine the content of reconstituting hematopoietic stem cells among human cells" (p. 529). In fact, the article by Lu et al., 1993, Blood 81:41-48 (reference DP) states that the hematopoietic repopulating ability of single human cord blood collections may be due at least in part to the presence of a population of cells, the high-proliferative potential colony-forming cells, first reported in this 1993 article to be present in human cord blood. Attention is further invited to the newspaper article in BioWorld Today, November 19, 1993, (reference FO), which states the following on page 4:

> The [cord blood cell] transplant procedure is only performed once, Kurtzberg [Joanne Kurtzberg, a professor of pediatrics at Duke University Medical Center in Durham, N.C.] explained, because it is such an aggressive treatment. And since there are no assays for stem cells *per se*, there's no way to know in advance if the cord blood actually contains sufficient stem cells to repopulate the bone marrow. The only way to determine this is to do the transplant.

Applicants submit that the evidence amply demonstrates that one skilled in the art, even with knowledge of the presence in cord blood of colony-forming cells and cells able to form colonies after replating, would <u>not</u> have reasonably predicted or expected with a reasonable expectation of success that such human cord blood cells would have utility for

hematopoietic reconstitution or for disorders treatable due to such utility, in the absence of Applicants' teachings.

Applicants further point out that use of blood collected from only a single neonate or fetus would be deemed necessary for therapeutic use of human neonatal/fetal blood[9], since combinations of collections from different neonates/fetuses would be avoided due to the danger of graft versus host disease and other problems stemming from histocompatibility mismatches, contamination by maternal cells, infectious agents, etc. (*see* discussion below for evidence that these concerns were rampant in the art). Indeed, the discussion of references presented below (subsection E(2)) demonstrates the necessity acknowledged in the art that sufficient repopulating cells be present in single cord blood collections for there to be an expectation of therapeutic utility, and the fact that there was no suggestion in the prior art, much less with a reasonable expectation of success, that sufficient long-term repopulating stem cells were present in single collections of human neonatal or fetal blood.

With respect to cryopreserved stem cells, the disclosures of the references cited by the Examiner regarding cryopreservation of stem cells are limited to bone marrow cells (Herzig) or adult peripheral blood leukocytes from cancer patients whose blood was "rebounding" after chemotherapy (see Herzig, pp. 133-134). Applicants emphasize that a teaching of the therapeutic utility of cryopreserved stem cells from particular sources cannot be deemed to suggest therapeutic utility of cryopreserved stem cells from other sources with any reasonable expectation of success, because, in addition to the reasons set forth above, cells from different sources are known to have different sensitivities to cryopreservation. Indeed, both fetal liver stem cells and fetal thymus stem

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9 Except in rare circumstances such as the case of collection from identical twin neonates or fetuses.

cells have been suggested to be significantly more sensitive to cryopreservation than bone marrow stem cells, and the art teaches away from therapeutic use of cryopreserved stem cells from fetal liver and fetal thymus. In this regard, the Examiner's attention is invited to O'Reilly et al., 1985, in *Fetal Liver Transplantation*, Gale R.P. et al., (eds.), Alan R. Liss, Inc., NY, pp. 327-342 (reference EB) at page 335, wherein it is stated that:

> A comparison of our results . . . and a review of the attributes of each approach has underscored several limitations to the use of fetal tissue transplants and led us to abandon this approach for all but exceptional cases.
>
> . . . . While large banks have been developed to cryopreserve fetal liver and thymus for transplantation purposes, to our knowledge, no human has ever been demonstrated to have been engrafted with cryopreserved fetal liver or thymus suggesting that these cells when cryopreserved may be considerably more fragile than their marrow counterparts.

Thus, the art cited by the Examiner does <u>not</u> suggest the therapeutic utility of cryopreserved human hematopoietic stem cells from sources other than bone marrow or possibly certain adult peripheral blood, much less provide a reasonable expectation of success at the claimed methods of treatment comprising introduction of human neonatal or fetal hematopoietic stem cells that have been cryopreserved or that are in combination with a cryoprotective agent. Thus, the claimed invention is nonobvious.

E. The Claimed Invention Presents Secondary Considerations That Are <u>Objective Evidence Of Nonobviousness</u>

As discussed *supra*, the following considerations are objective evidence of nonobviousness: long felt need, commercial success, failure of others, copying, and unexpected results. *E.g.*, <u>Avia Group Int'l Inc. v. L.A. Gear California, Inc.</u>, 853 F.2d 1557, 7 U.S.P.Q.2d 1548 (Fed. Cir. 1988); <u>Hybritech Inc. v. Monoclonal Antibodies,</u>

Inc., 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); In re Sernaker, 702 F.2d 989, 227 U.S.P.Q. 1 (Fed. Cir. 1983). In fact, the Court of Appeals for the Federal Circuit (CAFC) has consistently made clear that when evidence of such secondary considerations is present, it must be considered by the Examiner or a court in determining a question of obviousness. *See e.g.*, Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1379-80, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); Stratoflex Inc. v. Aeroquip Corp., 713 F.2d 1530, 1538-39, 218 U.S.P.Q. 871, 879 (Fed. Cir. 1983). "Indeed, evidence of secondary considerations may often be the most probative and cogent evidence in the record. It may often establish that an invention appearing to have been obvious in light of the prior art was not. It is to be considered as part of all the evidence . . . " Stratoflex Inc. v. Aeroquip, 713 F.2d at 1538-39, 218 U.S.P.Q. at 879. Such secondary considerations provide evidence that can both rebut a *prima facie* case of obviousness and demonstrate the nonobviousness of the claimed invention. *See e.g.*, In re Piasecki, 745 F.2d 1468, 223 U.S.P.Q. 785 (Fed. Cir. 1984).

The claimed invention displays secondary considerations which constitute objective evidence of nonobviousness, in particular, the fulfillment of long-felt but unresolved needs, initial skepticism expressed by experts in the art followed by widespread recognition and acceptance, achievement of unexpected results, and copying by others. These secondary considerations are clearly evidenced by the myriad publications in the art discussed hereinbelow, as well as the Second Bernstein Declaration.

(1) The Claimed Invention Fulfills Long-Felt Needs

It is well established case law that evidence of the satisfaction of a long recognized need and failures or difficulties encountered by those skilled in the art, are classical indicia of nonobviousness. In re Dow Chemical Co., 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988); Under Sea Indus. Inc. v. Dacor Corp., 833 F.2d 1551, 4 U.S.P.Q.2d 1772 (Fed. Cir. 1987); Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d. 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987). As described herein and in the Second Bernstein Declaration, Paragraphs 34-39, the claimed invention fulfills needs in the art which were recognized and persistent over a long length of time; and attempts to fulfill these needs had been repeatedly investigated by the art without satisfaction; and in contrast, the claimed invention of the instant application provides a method of treatment using a source of hematopoietic reconstituting stem cells with an ability to satisfy these needs that is superior to the alternatives which had been investigated by the art (see Second Bernstein Declaration, ¶ 34). Applicants submit that the long felt needs for methods of treatment by use of effective, abundant, inexpensive, safely and easily obtainable compositions for achieving hematopoietic reconstitution, and the difficulties encountered with methods disclosed in the prior art for use in hematopoietic reconstitution, provide further proof of the nonobviousness of the instantly claimed invention. The Second Bernstein Declaration, the many publications in the art that are discussed hereinbelow, and the specification of the instant application all evidence the long felt but unsolved needs, satisfied by the present invention, that rebut any *prima facie* case of obviousness and demonstrate the non-obviousness of the claimed invention. *See* In re Piasecki, 745 F.2d 1468, 223 U.S.P.Q. 785 (Fed. Cir. 1984).

Indeed, a source of stem cells capable of safely carrying out hematopoietic reconstitution, without problematic GVHD; with reduced potential for contamination by a

patient's malignant cells or for having other disorders or infections associated with an adult patient or with adult tissue;[10] easily obtainable (without entailing an invasive, surgical procedure with all its attendant costs and risks); inexpensive; abundant, and widely available; and not dependent on having a patient healthy enough to undergo the procurement procedure; has long been sought by those skilled in the art. The presently claimed invention affords each of these long-sought characteristics.

As discussed in Section 2 of the instant application, some years prior to the filing of the above-identified application, there existed recognition in the art that successful hematopoietic reconstitution would be valuable in the treatment or prevention of various diseases and disorders such as anemia, malignancies, autoimmune disorders, and other immune dysfunctions and deficiencies.

Dr. Bernstein, in the Second Bernstein Declaration, Paragraph 35, discusses the long-recognized drawbacks associated with the prior art method of bone marrow transplantation:

> The need for a safe, efficacious source of stem cells capable of effecting hematopoietic reconstitution has been recognized in the art since at least the 1950's, when bone marrow began to be studied for use in hematopoietic reconstitution, in order to reconstitute the hematopoietic system of cancer patients whose hematopoietic system had been destroyed by irradiation and/or chemotherapy. From the 1950's until the time the application leading to the '681 Patent was filed, bone marrow was the source of stem cells predominantly used in hematopoietic reconstitution, since no wholly satisfactory alternatives had been found. Bone marrow reconstitution has always suffered from the recognized drawbacks that collection of bone marrow cells is an invasive procedure, posing some risk to the donor, and is expensive and laborious. Additionally, autologous bone marrow transplantation has the long-recognized disadvantages of the sick or suboptimal condition of the donor, and the

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10 Cancer patients that undergo chemotherapy or radiation treatment form a substantial patient group in need of hematopoietic reconstitution; see references discussed hereinbelow.

> threat of marrow contamination with malignant cells in cases where the patient suffers from cancer. Allogeneic (non-autologous) bone marrow transplantation has the long-recognized disadvantages of the difficulty in finding a suitable (histocompatible) donor (necessary to avoid lethal GVHD), and the problem of GVHD occurrence even when using at least partially matched donors. Indeed, due to the heterogeneity of HLA antigens (the antigens which govern histocompatibility), it is often the case that suitable donors can't be found, or can't be found in time to avoid patient death. Even if suitable patients are registered in the National Marrow Donor Program (a registry of HLA-typed persons who volunteer to donate bone marrow to recipients who don't have a suitable sibling donor), death and illness can cause unavailability of the prospective donor when the need for his bone marrow has arisen. Locating, contacting, and counseling a donor is often time-consuming and expensive, as is the process of obtaining the donor marrow, forwarding it to the transplant center, and coordinating the hospitalization and collection. My personal experience has been that it often takes several months to find a suitable bone marrow donor for a patient with leukemia; oftentimes, that leukemia patient does not have several months to live. All of the foregoing disadvantages had been recognized in the art for over 20 years by the time the application leading to the '681 Patent had been filed.

(Second Bernstein Declaration, ¶ 35).

The instant application also notes the drawbacks with respect to the use of bone marrow. As stated on page 25, lines 25-27 of the instant application, collection is an invasive procedure, thus posing some risk to the donor, and is expensive and laborious: "[A]t present, the collection of bone marrow cells for transplantation is a traumatic experience which is costly in terms of time and money spent for hospitalization." Furthermore, with respect to attempted autologous hematopoietic reconstitution using bone marrow transplantation, it is noted that such entails many disadvantages not generally encountered with use of neonatal cells, including the danger of contamination with malignant cells and the sick or suboptimal condition of the donor (specification, page 11, lines 6-9; quoted below). Allogeneic (non-autologous) bone

marrow transplantation is noted to entail particular disadvantages. These disadvantages are described on page 11, lines 1-28 of the instant specification:

> Present use of bone marrow transplantation is severely restricted....Even in such an autologous system, the danger due to undetectable contamination with malignant cells, and the necessity of having a patient healthy enough to undergo marrow procurement, present serious limitations. [references]. Except in such autologous cases, there is an inevitable genetic mismatch of some degree, which entails serious and sometimes lethal complications. These complications are two-fold. First, the patient is usually immunologically incapacitated by drugs beforehand, in order to avoid immune rejection of the foreign bone marrow cells (host versus graft reaction). Second, when and if the donated bone marrow cells become established, they can attack the patient (graft versus host disease), who is recognized as foreign. Even with closely matched family donors, these complications of partial mismatching are the cause of substantial mortality and morbidity directly due to bone marrow transplantation from a genetically different individual.

In view of the foregoing long-recognized drawbacks, suitable alternatives to bone marrow as sources of stem cells with utility for hematopoietic reconstitution were searched for by the art. As described by Dr. Bernstein (Second Bernstein Declaration, ¶ 36), since at least the 1970's and into the 1980's, fetal thymus, fetal liver, and adult peripheral blood had been investigated as alternatives to bone marrow, as sources of stem cells with utility for human hematopoietic reconstitution, in the hope that they would overcome the recognized drawbacks associated with the use of bone marrow. However, the art did not consider any of these other sources to be satisfactory alternatives to the use of bone marrow at the time the priority application to the instant application was filed (Second Bernstein Declaration, ¶ 36). Dr. Bernstein describes the drawbacks associated with the use of fetal liver and fetal thymus as follows:

> Fetal thymus and fetal liver as sources of stem cells for hematopoietic reconstitution were found to suffer from the recognized drawbacks of high failure rates due to lack of long-term or complete hematopoietic repopulation and/or GVHD, as well as the evident problem of limited availability and accessibility. Fetal thymus and liver had to be surgically removed from aborted fetuses. Aborted fetuses could not be counted on to be available. Even if available, one could not count on obtaining a liver or thymus from a fetus old enough to have a liver or thymus large enough to afford a sufficient number of long-term marrow repopulating stem cells. These problems had led to the substantial rejection of the use of fetal thymus and/or liver for hematopoietic reconstitution by the time the application leading to the '681 Patent was filed.

(Second Bernstein Declaration, ¶ 37).

Dr. Bernstein describes the drawbacks associated with the use of adult peripheral blood as follows:

> Normal adult peripheral blood, due to its low levels of circulating stem cells, does not have any practical utility for effecting hematopoietic reconstitution, and thus was not deemed a practical alternative to bone marrow. Some success was achieved in the prior art in the use of adult peripheral blood mononuclear cells of certain leukemia patients; however, this success was largely viewed as due to the fact that the blood was collected while "rebounding" after chemotherapy (*i.e.*, while the stem and progenitor cell levels were believed to be increased in response to chemotherapy).[[11]] This procedure was thus recognized to suffer from the drawbacks of potential malignant contamination of the peripheral blood cells collected from the patient, as well the dependency of collection upon patient availability and condition (*i.e.*, involving collection from a patient with a sick or suboptimal condition) and limited as to when the cells could be collected at a time when the desirability of hematopoietic reconstitution for the particular patient was already recognized. It also involved intensive leucopheresis for collection of sufficient cells, which is time-consuming and expensive. Furthermore, the procedure suffered from variability in amount of long-term marrow repopulating stem cells in the collected blood, indicated by

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11 Dr. Bernstein refers by footnote here to Paragraphs 26-29 of the Second Bernstein Declaration.

> problems of incomplete or unstable hematopoietic repopulation presumably due to low levels of such cells.

(Second Bernstein Declaration, ¶ 38.)

Section 2.2 of the instant specification also notes that adult peripheral blood, fetal liver, neonatal spleen, and neonatal thymus have been investigated as possible sources of stem cells and other cells for hematopoietic reconstitution and found to have a number of drawbacks.

For example, with respect to the use of adult peripheral blood cells, it is noted that in adults, stem and progenitor cells are mostly confined to the bone marrow; very few circulate in the blood (specification, page 23, lines 29-30); and that it appears that while in some studies promising results have been obtained for patients with various leukemias and with lymphoma, other studies using peripheral blood have failed to effect reconstitution. Additionally, the collection of peripheral blood can be time consuming and uncomfortable. Furthermore:

> Many of the relative disadvantages discussed supra of the use of bone marrow cells for hematopoietic reconstitution [disadvantages due to age], also apply to the use of adult peripheral blood for such reconstitution, and thus, the use of neonatal cells for hematopoietic reconstitution according to the present invention provides distinct advantages over the employment of adult peripheral blood. It has been implied that the ability of autologous peripheral adult blood to reconstitute the hematopoietic system, seen in some cancer patients, is associated with the far greater numbers of circulating progenitor cells in the peripheral blood produced after cytoreduction due to intensive chemotherapy and/or irradiation (the rebound phenomenon) (To, L. B. and Juttner, C.A., 1987, Annot. Brit. J., Haematol. 66:285-288; see also 1987, Brit. J. Haematol. 67:252-253, and references cited therein). There are possible detrimental effects, known or unknown, of prior chemotherapy or irradiation, on the stem and progenitor cell populations found in these patients.

(specification, page 22, line 30 to page 23, line 13).

In contrast, the use of cryopreserved human neonatal/fetal blood stem cells overcomes these drawbacks long recognized in the art. This is described by Dr. Bernstein in Paragraph 39 of the Second Bernstein Declaration. As he explains:

> Cryopreserved human neonatal/fetal blood stem cells fulfill the long-recognized needs in the art for a source of stem cells (1) capable of safely and effectively carrying out human hematopoietic reconstitution, without severe GVHD; (2) with reduced potential for contamination by a patient's malignant cells or affliction with other disorders or infections associated with the patient or with adult tissue; (3) easily obtainable, without entailing an invasive surgical procedure with its attendant costs and risks; (4) not dependent on having a patient healthy enough to undergo the procurement procedure; (5) inexpensive; and (6) abundant/widely available. By way of example, placental and cord blood are available at <u>every birth</u> and were routinely discarded in the prior art. This great availability makes feasible the establishment of banks of cryopreserved neonatal/fetal blood stem cells, containing suitable donors for all population groups, even those minority populations presently underrepresented in the bone marrow registry, so as to expedite greatly finding and obtaining suitable donors of cells for hematopoietic reconstitution. Furthermore, frozen neonatal/fetal blood (or stem cell-containing fractions thereof) can be easily shipped and thawed for use, reducing the coordination, time, delay and expense associated with obtaining collected bone marrow. In addition, human neonatal/fetal blood has not been subjected to any detrimental effects associated with the aging process, for example, it should generally have a lower risk of containing infectious agents or disease than adult sources of long-term marrow repopulating stem cells. With respect to autologous use, the blood is collected at a time that is generally well prior to the onset of the illness (*e.g.*, cancer) desired to be treated by hematopoietic reconstitution, and can be stored for later use. In its simplest aspect, the human neonatal/fetal blood can be easily obtained by direct drainage from the umbilical cord, without the need for any invasive, surgical procedure or anesthesia. Numerous publications in the art have now appeared, documenting the clinical successes achieved using human cord blood in children suffering from various disorders, and demonstrating that cryopreserved human neonatal/fetal blood stem cells can serve as a source of long-term marrow repopulating stem cells that safely and effectively carry out hematopoietic reconstitution, without problematic GVHD.

(Second Bernstein Declaration, ¶ 39).

The instant specification also notes that in the use of neonatal or fetal cells as provided by the present invention, neonatal blood that is otherwise discarded (see specification, page 24, lines 1-2) is readily obtainable for use without risk to the donor. The neonatal/fetal blood cells can easily be obtained from umbilical cord blood available on delivery of the donor baby (and which blood is routinely discarded in the prior art). Specifically, cord blood can be obtained by direct drainage from the cord and/or by needle aspiration from the delivered placenta at the root and at distended veins (see specification, page 26, lines 22-26). Therefore, cord blood can be obtained without trauma to the donor, easily and inexpensively.

The instant specification also supplies the following description of additional long-desired advantages supplied by the use of human neonatal/fetal blood cells:

> Furthermore, the prospects of success in bone marrow transplantation decline with age; although it is not clear whether the age of donor or patient is more important, it is proper to infer that younger (neonatal) cells are preferable for hematopoietic reconstitution. Such neonatal or fetal cells have not been subjected to the "environmental outrage" that adult cells have undergone. Also, as an example of novel medical applications which may be feasible with neonatal cells but not with conventional bone marrow transplantation, restoration with self cells taken at birth can be valuable in the treatment of disorders such as declining immune responsiveness and autoimmunity (immune reactions against one's own tissues) which occur in increasing frequency with age.

(specification, page 22, lines 17-30).

> . . .
>
> There are additional reasons for preferring the use of neonatal cells for hematopoietic reconstitution as provided by the present invention. Neonatal blood is a preferred source of cells for hematopoietic reconstitution, since it is free from viral and microbial agents, known or unknown, latent or

> otherwise, that may be encountered in later life, other than those transmitted from the mother or during labor and delivery. In addition, in view of the extent to which the hematopoietic stem cell may possibly share with other cells the limitation in total number of cell divisions that it may undergo before senescence, it is proper to assume that the neonatal hematopoietic stem cell has a self-renewal and reconstituting capacity that is at least as great, and perhaps greater, than that of hematopoietic stem cells obtained at any later time in life.

(specification, page 23, lines 13-27).

The numerous publications listed below also documents the fact that sources of hematopoietic stem cells efficacious for hematopoietic reconstitution have long been sought as alternatives to bone marrow due to bone marrow's recognized disadvantages, but have not succeeded in satisfactorily fulfilling these long-felt needs in the art due to reduced efficacy in obtaining reconstitution and/or the inherent limitations in these alternative techniques.

Applicants also note that, in contrast to the prior art, use of the claimed methods thus far has been seen to entail unexpectedly low GVHD (as discussed hereinbelow). Many difficulties had been experienced in obtaining hematopoietic reconstitution using sources of stem cells in the prior art, particularly those sources investigated as alternatives to bone marrow with its recognized attendant disadvantages. In contrast, the methods of the present invention have been used to carry out the successful reconstitution of the hematopoietic system and thus treat disease in many different human patients.

Indeed, an abundance of publications in the relevant art attest to the fact that the need for the attributes supplied by the claimed invention was a recognized and persistent one over a long length of time, and that efforts in the prior art have been made over this length of time to solve these needs, but without the success afforded by the

claimed invention. A discussion of specific publications, discussed in chronological order, appears in Exhibit A, attached hereto.[12]

(2) Skepticism and Disbelief in the Art

Evidence that experts initially expressed skepticism upon learning of the invention, and evidence that experts subsequently praised its value, are both probative evidence of nonobviousness. *See e.g.* United States v. Adams, 383 U.S. 39, 43-44, 52 (1966); Corning Glass Works v. Sumitomo Electric U.S.A. Inc., 671 F. Supp 1369, 1398, 5 U.S.P.Q.2d 1545, 1569 (S.D.N.Y. 1987), aff'd, 868 F.2d 1251, 9 U.S.P.Q.2d 1962 (Fed. Cir. 1989) ("Praise for the invention, including awards accorded to the inventors for their invention, are further evidence of the novelty and worth of the inventions."); Jenn-Air Corp. v. Modern Maid Co., 499 F. Supp. 320, 326-27, 209 U.S.P.Q. 295, 301-02 (D. Del. 1980), aff'd, 659 F.2d 1068 (3d Cir. 1981) ("Articles commenting favorably upon a patented product may constitute evidence of a patent's validity in two ways . . . . Secondly, such articles may be direct technical evidence of non-obviousness"). "The skepticism of an expert, expressed before these inventors proved him wrong, is entitled to fair evidentiary weight . . . " In re Dow Chemical Co., 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1532 (Fed. Cir. 1988) *See also* Burlington Indus., Inc. v. Quigg, 822 F.2d 1581, 3 U.S.P.Q.2d 1436 (Fed. Cir. 1987) (a *prima facie* case of obviousness based on the prior art was rebutted by testimonial evidence that the invention met with initial incredulity and skepticism by experts); Environmental Designs, Ltd. v. Union Oil Co. of Calif., 713 F.2d 693, 697-98, 218 U.S.P.Q. 865, 869

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12 All the publications discussed in Exhibit A are believed either to be already of record or are being made of record in the Supplemental Information Disclosure Statement submitted concurrently herewith.

(Fed. Cir. 1983), cert. denied, 464 U.S. 1043 (1984) ("Expressions of disbelief by experts constitute strong evidence of nonobviousness.").

The publications discussed below are submitted as evidence of the clear skepticism and disbelief in the art as to the therapeutic utility of cryopreserved human cord blood stem cells, and thus evidence the lack of motivation in the prior art to therapeutically administer compositions comprising such cryopreserved cells. These references evidence such prior art skepticism and disbelief by showing the skepticism and severe doubt among those of ordinary skill in the art even after the initial publication by the instant inventors disclosing the idea of the instant invention! These references show chronologically the transition in the thinking in the art, from skepticism to uncertainty to an expectation of utility of the claimed invention, which transition occurred due to the publications in the field which gradually demonstrated, irrefutably, by actual successful therapeutic uses *in vivo*, the human therapeutic utility of the claimed methods. These references also further document the reasons stated and explained above, as to why one of ordinary skill in the art would not have reasonably expected or predicted the therapeutic utility of cryopreserved human neonatal/fetal blood stem cells, in view of the teachings of the references cited by the Examiner. These references, and the evidence thus presented, are discussed in chronological order below, from the earliest to the most recently published articles.

Broxmeyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:3828-2832 (reference BC) is a publication by the inventors et al. which reports, based on an analysis of numerous cord blood collections from single individuals, that the number of progenitor cells present in such collections fell within the range reported for successful engraftment by bone marrow stem cells and discloses the prospect of storing cord blood cells for future therapeutic use.

Linch and Brent, Nature 340:676 (reference DM) evidence the skepticism in the art <u>even after</u> the publication by Broxmeyer et al., reference BC. With respect to the data reported by Broxmeyer et al. in reference BC on the volume of cord blood that may be extracted, which Linch and Brent acknowledge is "surprisingly high," Linch and Brent calculate the number of progenitor cells thus expected to be present, and state that this amount "should be sufficient for reconstitution after allogeneic transplantation <u>provided that the stem-cell/progenitor-cell ratio is not appreciably less than in adult bone marrow</u>" (emphasis added), and add that "[o]nly clinical studies can prove this point." Linch and Brent then describe the "two possible difficulties with the strategy proposed by Broxmeyer et al., both relating to the possible development of graft-versus-host (GVH) disease": (1) depletion of T lymphocytes to minimize GVH may result in unacceptable losses of progenitor cells; and (2) contamination of neonatal blood by maternal cells could cause GVH disease. The entire tone of the article is one of strong doubt in the utility proposed by the instant inventors.

Gluckman et al., 1989, New Engl. J. Med. 321:1190-1191 (reference CE) is a publication by the <u>inventors</u> et al. which reports the successful hematopoietic reconstitution of a child with severe Fanconi's anemia though the administration of cryopreserved umbilical cord blood from his HLA-identical sister, and states that "umbilical-cord blood can be considered an efficacious source of sufficient cells for clinical hematopoietic reconstitution" (page 1178, column 1).

Nathan, 1989, N. Engl. J. Med. 321(17):1190-1191 (reference HM) is an article written in view of O'Reilly et al., 1984, Sem. Hematol. 21(3):188-221 (reference EA), which again evidences the extant skepticism and doubt as to the utility of the claimed invention, even after the first clinical success. Nathan acknowledges, as he must in view of the evidence of reference EA, that successful engraftment has been achieved

with umbilical cord blood, but still expresses strong doubt with respect to the utility of the claimed invention for instances other than the "rare occasions" on which cord blood can be used "to save the life of an older sibling." As Nathan states:

> It should be emphasized, however, that the recipient was a small child and that the number of nucleated cells infused was not as high as one would like to see in most transplants of allogeneic marrow. As has been pointed out by others, the technique has several other limitations, including a higher risk of graft-versus-host disease due to interaction of the T cells of the fetus with those of the mother. Thus, although this is an interesting use of a substance usually discarded, it is unlikely to have broad application.

Gluckman et al., 1990, Bone Marrow Transplant 5 (Suppl. 2):42 (reference CF) (of which co-inventor Broxmeyer is a co-author) report the successful hematopoietic reconstitution of two patients with Fanconi's anemia, each of whom received the thawed cord blood cells of an HLA-identical sibling. It is stated, however, that before the technique can be generalized, "improvement of collection and cell purification, studies of the maternal contamination and the function of both myeloid and lymphoid cells are necessary."

Auerbach et al., 1990, Transfusion 30(8):682-687 (reference AW) (of which two of the instant inventors are co-authors) report the successful use of cord blood from a single individual to effect hematopoietic reconstitution in two patients with Fanconi's anemia, and suggest the therapeutic application of cord blood to other diseases treatable by bone marrow transplantation and the establishment of a bank of cryopreserved cord blood cells for transplantation (page 686, column 1). The authors acknowledge the doubts expressed by others with respect to therapeutic utility, stemming from concerns of maternal cell contamination of cord blood and the onset of graft-versus-host disease (GVHD) (page 686, columns 1-2). With respect to these concerns, it is stated that the authors' laboratory tests did not detect any maternal cell contamination

although further tests were in progress, and that two Fanconi's anemia patients who received cord blood had less GVHD than was the norm. Another concern is also acknowledged: "whether there will be enough stem or progenitor cells from a single cord blood collection to repopulate the hematopoietic system of an adult;" with respect to this concern, it is stated that collections of cord blood have been made which contain much greater numbers of progenitor cells than that used successfully for the patients with Fanconi's anemia.

Broxmeyer et al., 1990, Int. J. Cell Cloning 8(Suppl. 1):76-91 (reference BA) is a publication by the inventors et al. The authors discuss their studies on the number of progenitor cells present, both before and after cryopreservation, in single cord blood collections. The authors state that cord blood <u>from a single individual</u> should provide sufficient reconstituting cells for effective hematopoietic repopulation of an autologous or HLA-compatible allogeneic recipient (page 77), based on the successful use of HLA-matched sibling cord blood for reconstitution. The use of cord blood for treatment of conditions treated by bone marrow transplantation is suggested (page 83), and concerns (GVHD and maternal contamination) previously vocalized by others are addressed. The authors were "struck by the mild or absent GVHD apparent in the two patients with Fanconi's anemia transplanted with umbilical cord blood cells." With respect to maternal cell contamination, the authors state:

> "[a] concern expressed by others [citation] in response to our scientific report [citation], but prior to publication of our clinical study [citation], is the possibility of contamination of cord blood by maternal cells. Our initial laboratory investigation did not detect . . . contamination of cord blood cells with maternal cells, but certainly this possibility needs to be one of a number of considerations that are addressed. . . . " (p. 85)

Attention is also invited to the "Discussion" section of this article, particularly on page 90 wherein O'Reilly questions Broxmeyer with respect to concerns of GVHD and maternal cell contamination, stating, for example, "Anyone who's been in the delivery room at the time when a baby is born, recognizes often that the cord can be bloody from the mother's blood. . . . If it [the collected cord blood] does happen to contain the mother's blood, you're in trouble with GVH in some instances."

Broxmeyer et al., 1991, Blood Cells 17:313-329 (reference BF) is a publication by the inventors et al. which reports the successful cord blood transplant of three patients with Fanconi's anemia (and one unsuccessful transplant, see page 325), and discloses that a cord blood transplant was done by others for a patient with juvenile chronic myelogenous leukemia. The authors state that it was their feeling that cord blood transplants should be used for conditions that call for bone marrow transplantation (page 320). The authors then discuss in detail the skepticism and questions expressed by others in editorials written in response to both their laboratory and clinical studies, which questions have focused on (a) whether the presence of maternal cell contamination will cause clinical problems, (b) questioning of the broadness of applicability of cord blood transplantation, and (c) whether, due to low immunological reactivity, more mismatched transplants may be possible using cord blood than with bone marrow cells (page 321-324). With respect to (a), it is stated that in the two cases of Fanconi's anemia treated with cord blood, GVHD was mild; furthermore, tests were not able to detect maternal cell contamination. With respect to (b), it is stated that questions of others have focused on whether other disorders treated by bone marrow transplantation can be treated with cord blood, and whether cord blood can be used to transplant adults (since the number of cells from a single collection available for transplantation may be limiting);

with respect to both these issues, the authors state it is their feeling that both can be accomplished. (c) is stated to be an intriguing possibility.

McGlave, 1991, Blood Cells 17:330-337 (reference DS) is a commentary written on the foregoing reference BF by the inventors et al., finally acknowledging that reference BF summarizes a series of *in vitro* and *in vivo* observations by Broxmeyer (an instant inventor) and colleagues demonstrating the feasibility of the use of human cord blood for allogeneic transplantation. While still reiterating the skepticism regarding the ability to obtain engraftment in large recipients or more unrelated recipients (page 332), McGlave states that cord blood cells could at present be used to treat other lethal hematopoietic disorders and discusses how harvesting, typing, and preservation of cord blood may be justified if further studies support cord blood use for unrelated transplantation. The author concludes that "the studies presented by Broxmeyer present an exemplary model of beneficial clinical therapy evolving from a good idea, animal research, and subsequent *in vitro* studies of human hematopoietic progenitors" (page 333).

Pollack et al., 1991, Hum. Immunol. 30(1):45-49 (reference EE) report that it has been demonstrated that human cord blood from HLA-matched donors can provide sufficient numbers of progenitor cells for hematopoietic reconstitution, and state the idea of freezing cord blood for subsequent therapeutic use.

Broxmeyer et al., 1992, Proc. Natl. Acad. Sci. USA 89:4109-4113 (reference BE) (of which co-inventor Broxmeyer is a co-author) disclose the many types of patients which had by then been the recipients of cord blood transplants. The authors state that since progenitor cell CFU-GM colony forming assays showed that <u>single</u> cord blood collections contained numbers of CFU-GM similar to that used for successful bone marrow transplantation, this suggested to the authors (of which inventor Broxmeyer is included) that sufficient cells were present in cord blood for hematopoietic engraftment of

an adult although "of course" clinical verification was required (pages 7-8). With respect to the concerns expressed by others, although "[m]aternal cell contamination had been considered an issue . . . thus far this does not appear to be a problem . . . " (page 9). The "increasing number of centers performing cord blood transplants" and the fact that people "have already begun to seriously consider the establishment of cord blood banks" is noted (page 10).

Schaison, 1992, Bone Marrow Transplantation 9(Suppl. 1):93-94 (reference ET) is a reference that acknowledges the numerous benefits of using cord blood stem cells therapeutically, and considers the question of frozen cord blood banking.

Alby, 1992, Bone Marrow Transplantation 9(Suppl. 1):95-96 (reference AU) also acknowledges the benefits of using cord blood for reconstitution ("BMT with cord blood is a main progress sparing precious months"), while discussing some of the societal and psychological concerns.

Gluckman et al., 1992, Bone Marrow Transplantation 9 (suppl. 1):114-117 (reference CC) disclose that patients with various hematological diseases, including malignant and nonmalignant disorders have now been successfully transplanted with cord blood, and acknowledge cord blood's utility for treating diseases in addition to Fanconi's anemia. The authors state that their results show that the number of cells obtained from a <u>single</u> cord blood collection is not a limiting factor, state that the problem of maternal contamination has been ruled out by their tests on cord blood-transplanted patients, propose cord blood collection and storage, and acknowledge the possibility of using cord blood in partially mismatched[13] situations and for gene therapy.

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[13] Partial mismatch refers to partial mismatch of the histocompatibility antigens (of which there are six). "HLA-identical" persons are totally matched, in all six antigens.

Wagner and Broxmeyer, 1992, Blood 80:1624 (reference FU) (of which co-inventor Broxmeyer is a co-author) state that the recent clinical successes with umbilical cord blood transplantation have generated considerable interest in techniques for umbilical cord and placental blood collection and that the placental blood volume after cord clamping has often been grossly underestimated by obstetricians, most of whom have little experience with collecting cord blood other than where small volumes are required. This publication shows that the art was not familiar with the volumes of neonatal blood obtainable from single individuals (which in turn determine in part the numbers of stem and progenitor cells available).

Wagner et al., 1992, Blood 79:1874-1881 (reference FS) (of which co-inventor Broxmeyer is a co-author) disclose that umbilical cord blood from an HLA-identical sibling contains sufficient numbers of hematopoietic stem cells to engraft a leukemia patient, and that there was no detectable maternal cell or bacterial contamination or GVHD. The authors state that in view of their clinical results and those of others, human cord blood should be viewed as an alternative source of hematopoietic stem cells (page 1879, column 2), and suggest the routine collection and storage of umbilical cord blood.

Broxmeyer et al., 1992, Proc. Natl. Acad. Sci. USA 89: 4109-4113 (reference BE) (of which co-inventor Broxmeyer is a co-author) disclose experiments which suggested that cord blood contains a larger number of early myeloid progenitor cells (MPC) than previously recognized, and that there are sufficient numbers of cells in a single cord blood collection to engraft an adult. In addition, the results suggested the possibility that cord blood may contain greater numbers of an earlier, more immature subset of MPC than found in adult bone marrow.

Vilmer et al., 1992, Transplantation 53(5):1155-1157 (reference FQ) demonstrate the feasibility of using HLA mismatched cord blood to transplant a patient with advanced leukemia, and state that there was no maternal cell contamination.

Hows et al., 1992, The Lancet 340: 73-76 (reference CU) acknowledge the use of cord blood for transplantation of children with leukemia or Fanconi's anemia, and report, based on their colony forming assays, that single cord blood donations may also be sufficient to engraft adults having leukemia or other hemopoietic disorders. Cord blood banking is proposed.

Cairo et al., 1992, Pediatric Res. 32:277-281 (reference BH) acknowledge that the recent clinical success using cord blood to reconstitute a patient with Fanconi's anemia has sparked renewed interest in neonatal hematopoiesis (page 277), and state that cord blood contains enough early and committed progenitor cells for allogeneic reconstitution (page 280).

Lu et al., 1993, Blood 81:41-48 (reference DP) (of which co-inventor Broxmeyer is a co-author) identify a population of high-proliferative potential colony-forming cells (HPP-CFC) in umbilical cord blood, and suggest that these cells may at least in part be responsible for the ability of single collections of umbilical cord blood to clinically engraft the hematopoietic systems of recipients.

Rubinstein et al., 1993, Blood 81:1679-1690 (reference EQ) suggest, as warranted and justified in view of the clinical successes and benefits of using cord blood for hematopoietic reconstitution, the use of banked placental blood to carry out systematic studies of the feasibility of using such blood for "bone marrow" reconstitution of unrelated recipients on a large scale. Concerns affecting the feasibility of using placental blood in such manner are discussed, including maternal cell contamination which is stated not to have been found upon the authors' testing of cord blood collections.

Newton et al., 1993, Exp. Hematol. 21:671-674 (reference DY) state that, in view of the clinical success and suggestion of use in unrelated patients of cord blood transplants, the widening indications for cord blood transplantation would justify the establishment of HLA-typed cord blood banks (of frozen cord blood). It is stated that the therapeutic utility of cord blood appears to be related both to its high content of stem and progenitor cells, and progress in increasing volume of single cord blood collections (page 671).

Ballantyne, 1993, "A Cord Linking Life and Life", The Times, May 6, 1993 (reference AY) is a newspaper article that reports and discusses the hope that cord blood banks will be established and be able to provide, within the next 2-3 years, cord blood for transplantation of thousands of children and adults.

Biotechnology Business News, 1993 (reference AQ); Jaroff, May 31, 1993, Time Magazine, (reference CW); and SCRIP, June 15, 1993, p. 26 (reference AR) are newspaper or magazine articles, all of which report novel gene therapy trials using recombinant cord blood stem cells to repopulate the hematopoietic system of children with severe combined immunodeficiency.

Moritz et al., 1993, J. Exp. Med. 178:529-536 (reference DW) acknowledge that human cord blood has been demonstrated to be capable of reconstituting the lympho-hematopoietic system in transplant protocols. The authors note that "no direct in vitro assay is available to determine the content of reconstituting hematopoietic stem cells among human cells" (page 529). The authors state that although there is no such direct *in vitro* assay, "multiple investigators have now demonstrated the capacity of single CB [cord blood] collections to reconstitute the lympho-hematopoietic system of infants and children. . . . data suggest that single CB samples may also be sufficient to reconstitute hematopoiesis in adult recipients" (emphasis added; pages 529-530).

Kohli-Kumar et al., 1993, Brit. J. Haematol. 85:419-422 (reference DF) (of which co-inventor Broxmeyer is a co-author) reports the successful hematopoietic reconstitution of a child with Fanconi anemia by use of HLA-matched sibling umbilical cord cells. Full marrow engraftment, with no residual host cells, was documented. There was no GVHD, and it is stated that cord blood transplants have been associated with a low incidence of GVHD (page 421, column 1). It is also stated that studies have not substantiated the concern regarding maternal cell contamination (page 421, column 1). Cord blood banks are reported to be a future option, "providing a ready source of stem cells available for transplant and making use of a resource otherwise wasted" (page 421, column 2).

Wagner et al., 1993, Blood 82(10) Suppl. 1: Abstr. 330 (reference FT) (of which co-inventor Broxmeyer is a co-author) describes the results of umbilical cord blood transplantation in 26 children with malignant or nonmalignant disorders. Eighteen patients showed donor cell engraftment, four failed to engraft, two died too early to evaluate, and two were not yet evaluated for engraftment. It is stated that the "data demonstrate that umbilical cord blood is an acceptable source of transplantable pluripotential hematopoietic stem cells with low GVHD potential."

Van Brunt, November 19, 1993, BioWorld Today, (reference FO) is a newspaper article reporting the establishment of a storage bank of umbilical cord and placental blood, for future use in therapy.

Vowels et al., 1993, New Engl. J. Med. 329:1623-1625 (reference FR) reports the case of a boy with X-linked lymphoproliferative disease in whom the transplantation of cryopreserved cord blood from an HLA-identical sibling resulted in hematopoietic and lymphopoietic reconstitution, with a correction of the genetic defect underlying the disease and a correction of his hypogammaglobulinemia.

(3) Copying of the Claimed Invention by Others Demonstrates its Nonobviousness

Copying is evidence of nonobviousness. *See e.g.*, Diamond Rubber Co. v. Consolidated Rubber Tire Co., 220 U.S. 428, 441 (1911); Avia Group Int'l Inc. v. L.A. Gear California, Inc., 853 F.2d 1557, 1564, 7 U.S.P.Q.2d 1548, 1554 (Fed. Cir. 1988) ("Copying is additional evidence of nonobviousness.").

Copying of the claimed invention by others due to its striking efficacy and/or advantages (relative to the prior art) in its therapeutic use in hematopoietic reconstitution of humans attests to its nonobviousness. As the authors of Vilmer et al., 1992, Transplantation 53(5):1155-1157 (reference FQ) state regarding their cryopreservation and use of cord blood for hematopoietic reconstitution (p. 1155, col. 1): "Encouraged by the favorable results with HLA-matched cord-blood transplantation in Fanconi's anemia (3) [Gluckman, E., Broxmeyer, H.E., Auerbach, A.D. et al., 1989, N. Engl. J. Med. 321:1174 (II), this procedure was considered in our patient whose mother was pregnant, after autologous bone marrow had been cryopreserved." Vilmer et al. copy the claimed invention due to its superior utility, even when autologous bone marrow was available for use. Vowels et al. 1993, N. Engl. J. Med. 329:1623-1625) (reference FR) employ transplantation of cord blood, "cryopreserved by the method of Broxmeyer et al. [citing a publication by two of the instant co-inventors, Drs. Broxmeyer and Douglas, et al.; 1989, Proc. Natl. Acad. Sci. USA 86:3828-3832 (reference BC)," to correct a genetic defect in a boy, in spite of the fact that a matched unrelated bone marrow donor had been identified (p. 1623, col. 2, third full paragraph). Kohli-Kumar et al., 1993, Brit. J. Haematol. 85:419-422 (reference DF) also reports the cryopreservation of cord blood and its successful use for hematopoietic reconstitution, thus copying applicants' claimed invention. The following manuscripts also report the cryopreservation of

placental or cord blood, or a stem cell-containing fraction thereof, and its successful use for hematopoietic reconstitution, thus copying the applicants' claimed invention: Kurtzberg et al., 1994, "The use of umbilical cord blood in mismatched related and unrelated hemopoietic stem cell transplantation," Blood Cells 20(2-3):275-283; Vowels et al., 1994, "Use of granulocyte-macrophage colony stimulating factor in two children treated with cord blood transplantation," Blood Cells 20(2-3):249-254; Issaragrisil, 1994, "Cord blood transplantation in thalassemia," Blood Cells 20(2-3):259-262; Kernan et al., 1994, "Umbilical cord blood infusion in a patient for correction of Wiskott Aldrich Syndrome," Blood Cells 20(2-3):245-248; Thompson, 1995, Science 268:805-806 (reference IB).

Furthermore, banks of cryopreserved cord blood, for eventual use in hematopoietic reconstitution have been established. Van Brunt, Nov. 19, 1993, BioWorld Today, (reference ID) reports the establishment of a (frozen) cord blood bank. Kurtzberg et al., 1994, cited *supra*, discloses the use in two patients of cryopreserved cord blood obtained from a blood bank. The abstract by Broxmeyer et al. (reference GO), entitled "Cord blood transplantation: an update," presented August, 1994, at the Meeting of the International Society for Experimental Hematology, also reports the establishment of a cord blood bank, from which cryopreserved blood has been obtained and used in three unrelated transplants.

(4) Use Of The Claimed Invention Achieves Surprising Results

Use of the claimed invention achieves surprising or unexpected results, providing further objective evidence of nonobviousness. The ability to collect a volume

of human neonatal/fetal blood from a single individual[14] that contains sufficient long-term marrow repopulating stem cells to carry out hematopoietic reconstitution of a human[15] was unexpected. Attention is particularly invited to the discussion of Linch and Brent, 1989 (reference DM) who acknowledge that the volume of cord blood that may be extracted (as reported by the inventors et al. in Broxmeyer et al., 1989, Proc. Natl. Acad. Sci. USA 86:3828-3832 (reference BD) is "surprisingly high" (p. 676). Wagner and Broxmeyer, 1992, Blood 82:1624 (reference FU) also note that "[t]he volume of blood that remains in the placenta after clamping the umbilical cord has often been grossly underestimated by obstetricians."

Furthermore, the relatively low incidence of GVHD[16] accompanying the use of the claimed invention for hematopoietic reconstitution in humans was unexpected. In contrast to prior art methods, the use of the claimed invention has thus far achieved a surprisingly low incidence of GVHD and significant success in hematopoietic reconstitution of humans (acknowledged by those skilled in the art). This is evidenced by the discussion of the cited references above. Particular attention is invited to Nathan, 1989, N. Engl. J. Med. 321(17)1190-1191 (reference HM) this reference demonstrates that in contrast to the <u>low</u> incidence of GVHD actually displayed in the use of the claimed

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[14] As demonstrated by the publications discussed above, one of ordinary skill in the art believed that in order for the claimed invention to have utility, sufficient reconstituting stem cells would need to be present in a single collection of human cord blood (in order to avoid the significant threat of GVHD (or graft vs. graft disease) if collections from different individuals were used. (Note, however, that the application teaches that expansion methods may reduce the amount needed to be present.)

[15] The specification teaches that single collections of human neonatal/fetal blood contain sufficient stem cells to carry out hematopoietic reconstitution of a human (see Section 6.8).

[16] The low incidence of GVHD need not be expressly disclosed in the specification, since it inherently flows from the use of the claimed composition as disclosed. *See* <u>In re Slocombe</u>, 510 F.2d 1398, 1403, 184 U.S.P.Q. 740, 743 (C.C.P.A. 1975); <u>In re Khelghatian</u>, 364 F.2d 870, 876, 150 U.S.P.Q. 661, 666 (C.C.P.A. 1966); <u>In re Zenitz</u>, 333 F.2d 924, 927-28, 142 U.S.P.Q. 158, 161 (C.C.P.A. 1964); <u>Ex parte Sasajima</u>, 212 U.S.P.Q. 103, 104-105 (P.T.O. Bd. App. 1980).

invention, it was expected that the use of the claimed invention for hematopoietic reconstitution would entail a higher risk of GVHD than other methods. As Nathan states (p. 1190, col. 2, emphasis added):

> It should be emphasized, however, that the recipient was a small child and that the number of nucleated cells infused was not as high as one would like to see in most transplants of allogeneic marrow. As has been pointed out by others, the technique has several other limitations, including a higher risk of graft-versus-host disease due to interaction of the T cells of the fetus with those of the mother. Thus, although this is an interesting use of a substance usually discarded, it is unlikely to have broad application.

Attention is also invited to the Discussion section at p. 90 of Broxmeyer et al., 1990, Int. J. Cell Cloning 8(Suppl. 1):76-91 (reference BA) wherein O'Reilly asks whether the use of cord blood for hematopoietic reconstitution entailed any GVHD, and then expresses his concern regarding GVHD:

> O'Reilly: Anyone who's been in the delivery room at the time when a baby is born, recognizes often that the cord can be bloody from the mother's blood. You would want to be sure that when you're collecting the blood, it isn't the mother's cells that you're seeing in the lymphoid fraction. If it does happen to contain the mother's blood, you're in trouble with GVH in some instances.

Similarly, Vilmer et al., 1992, Transplantation 53(5):1155-1157 (reference FQ) "[h]uman umbilical-cord blood as a source of transplantable stem cells was proposed for HLA matched allogeneic reconstitution although some authors have stressed the potential high risk of graft-versus-host disease [citations]" (p. 1155, col. 1). The authors note, after reporting their successful use of cord blood for hematopoietic reconstitution, that ""[t]he statement that cord-blood cells are contaminated by maternal cells and therefore have the potential for inducing GVHD [citation] is not confirmed by this observation" (p. 1156, col. 2), and that "[i]t is noteworthy that the HLA disparity, a

significant risk factor for graft failure and for GVHD, did not contribute to the development of such complications" (p. 1157, col. 1).

Also, Hows et al., 1992, The Lancet 340:73-76 (reference CU), unexpectedly find that "the quality and quantity of HUC [human umbilical-cord]-blood-derived hemopoietic 'stem' cells are better than those of normal bone marrow" (p. 73, col. 1), based on, *inter alia*, their findings that the amplitude and length of progenitor cell production from cord blood in long-term culture is superior to those of normal bone marrow (p. 75, col. 2). They also report that "[t]he fear that graft-versus-host disease may arise from mismatched maternal lymphocytes [citation] contaminating HUC blood appears unfounded since severe graft-versus-host-disease has yet to be reported after HUC-blood transplants [citations]" (p. 75, col. 2).

Socié et al., 1994, Blood 83:340-344 (reference HT), also notes the concern engendered by the potential for maternal cell contamination of cord blood upon the initial disclosure of the invention, and that this concern has subsequently been observed to be unfounded:

> However, already by 1989, two editorials pointed out that, even if one cord blood can be sufficient to allow reconstitution after allogeneic transplantation, its use might be hampered by contamination of neonatal blood with maternal cells [citations]. This possibility was raised because some blood leakage from fetus/neonate to the mother occurs in up to 50% of births, usually during parturition, and, occasionally, clinically significant leakage occurs from mother to fetus. The incidence of relatively minor leakages from maternal to the fetal circulation during parturition is not known, but was assumed to be high [citation]. The magnitude of maternal blood contamination could be increased by the collection procedure, which tries to collect optimal amounts of blood. More importantly, mature maternal T lymphocytes incompletely matched with the histocompatibility antigens of the recipient could contribute unacceptably to graft-versus-host disease (GVHD) after transplant [citation]. Graft-versus-host disease has been limited or absent thus far in the small number of children

> transplanted with cord blood [citation], but all except one [citation], had received a matched sibling transplant . . .

(p. 340-41).

The authors then use the sensitive technique of DNA amplification, to investigate more rigorously if maternal cell contamination occurs, and found that "maternal cell contamination of neonate blood is a very rare event and of a low magnitude when it occurred" (p. 341, col. 1, first paragraph).

Additional publications discussed hereinabove further evidence that GVHD was initially a strong concern, *i.e.*, problematic GVHD was expected, due to the threat of maternal cell contamination, but that unexpectedly, the use of the claimed invention for hematopoietic reconstitution has involved relatively low GVHD. See, in particular, Broxmeyer et al., 1990, Intl. J. Cell Cloning 8(Suppl. 1):76-91 (reference BA) and Broxmeyer et al. 1991, Blood Cells 17:313-329 (BF) regarding acknowledgment of concerns regarding GVHD and the fact that patients who received cord blood for hematopoietic reconstitution had strikingly <u>less</u> GVHD than was the norm. *See also* Kohli-Kumar et al., 1993, Brit. J. Haematol. 85:419-422 (reference DF) stating that cord blood transplants have been associated with a low incidence of GVHD (p. 421, col. 1) and that studies have not substantiated the concern regarding maternal cell contamination (p. 421, col. 1). Wagner et al., 1993, Blood 82(10)(Suppl. 1):Abstr. 330 (reference FT), also discloses the "low GVHD potential" of cord blood stem cells. Rubinstein et al., 1993, Blood 81:1679-1690 (reference EQ), also state that maternal cell contamination, with its "potentially dire consequences," has not been found upon the authors' testing of cord blood collections (p. 1681, cols. 1-2), and that the "sibling-donor placental blood transplants performed up to now suggest that allorecognition and GVHD may be less intense than in recipients of similarly compatible BM [bone marrow]" (p. 1682, col. 1).

The HLB Newsletter, June 23, 1994, Vol. 10, No. 9 (reference GF), reports that a draft initiative received by the National Heart, Lung, & Blood Institute, which was viewed "with its highest enthusiasm," states that "Studies of over 40 cord blood transplants performed so far for a number of malignant [citing examples] and nonmalignant [citing examples] diseases in children suggest that graft vs. host disease has been very low with this source of transplantable cells." (p. 73, col. 1). Wagner, 1994, "Umbilical cord blood transplantation, overview of the clinical experience," Blood Cells 20(3):227-233 (reference IF), notes, in reviewing data of 34 cord blood transplants reported to the International Cord Blood Transplant Registry, that GVHD occurred with low frequency; in particular, of 23 evaluable patients with HLA-identical or HLA-1 antigen mismatched donors, none suffered from grade 2-4 acute GVHD.[17]

F. The Examiner's Rejection Involves The Improper Use Of Hindsight

Applicants respectfully submit that the remarks made hereinabove clearly demonstrate the nonobviousness of the instant invention; there was no motivation in the art to use cryopreserved human neonatal/fetal blood stem therapeutically, much less a reasonable expectation of success in so doing. There was no reasonable expectation that human neonatal or fetal blood contained cells with therapeutic utility for hematopoietic reconstitution and thus disorders amenable to treatment by hematopoietic reconstitution. This is shown by the lack of identity acknowledged in the art between stem cells from different sources; the lack of identity acknowledged in the art between the hematopoietic reconstituting cell and a cell with the ability to form colonies *in vitro* (or even cells with

[17] *See also* McGlave, 1991, Blood Cells 17:330-337 (reference DS); Broxmeyer et al., 1992, Proc. Natl. Acad. Sci. USA 89:4109-4113 (reference BE) at p. 9; Gluckman et al., 1992, Bone Marrow Transp. 9(suppl. 1):114-117 (reference CC) at p. 115; Wagner et al., 1992, Blood 79:1874-1881 (reference FS) at p. 1879, second full paragraph.

the ability to form colonies which can be replated to form secondary colonies); the lack of suggestion or expectation that sufficient stem cells would be present in a collection of neonatal/fetal blood from a single individual to effect hematopoietic reconstitution; the lack of suggestion or expectation that sufficient stem cells in a human neonatal/fetal blood collection, assuming *arguendo* such were present, would survive cryopreservation; and the doubt that human neonatal/fetal blood stem cells could be useful for *in vivo* reconstitution due to the expected presence of GVHD and maternal cell contamination. To conclude otherwise is to use impermissible hindsight. In view of the foregoing, Applicants submit that they have demonstrated that the claimed methods are not obvious, in view of the deficiencies of teachings of the references relied upon by the Examiner.

5. The Examiner's Rejection for Obviousness-Type Double Patenting

Claims 60-62, 67-102 and 104-111 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 13-35, 47-53 and 57 of U.S. Patent No. 5,192,553.

In response, Applicants submit that a terminal disclaimer will be filed either by supplemental Amendment or upon the indication by the Examiner of allowable subject matter.

CONCLUSION

Applicants respectfully request that the present response be entered and made of record in the instant application. Withdrawal of the Examiner's rejections and

an early allowance is earnestly requested. If any issues remain in connection herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same.

Respectfully submitted,

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